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14. ABSTRACT

Cancer cells evade immunity during malignant progression. Thus, strategies to reverse this process could offer new therapeutic options. Overexpression of indoleamine 2,3-dioxygenase (IDO) in cancer cells inhibits T cell activation by tumor antigens. Notably, combining an IDO inhibitor with cytotoxic drugs such as paclitaxel strongly and safely enhances antitumor efficacy in animal models of breast cancer. In this project, we proposed to test whether combining an IDO inhibitor with paclitaxel could safely enhance efficacy against prostate tumors. The suggested model system was a variant of the TRAMP mouse engineered with a prostate-specific luciferase transgene. Briefly, our objectives were to assign tumor-bearing mice to control and drug treatment groups and to compare tumor response after control or experimental therapies by bioluminescence imaging. Unfortunately, a fatal pitfall prevented the use of the model (nonselective luciferase expression). Two alternate models explored – each based on engraftment of luciferase-expressing cells into the prostate or under the skin – also exhibited fatal pitfalls (arising tumors tended to spontanously regress). Our findings raised serious concerns about the utility of luciferase-expressing prostate tumor cells for bioluminescence-based studies of prostate cancer pathophysiology and therapeutic response.

15. SUBJECT TERMS
experimental chemotherapeutics, immunomodulation, mouse model

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INTRODUCTION

Immunity is important to limit cancer progression. Genes that modulate immune recognition may influence cancer susceptibility or prognosis and their study may cue novel strategies to stimulate tumor immunity. BAR adapter proteins encoded by the Bin1 gene have been implicated in the regulation of vesicle trafficking processes but also in nuclear events. Initial studies of Bin1 were based on its ability to interact with and inhibit the oncogenic properties of c-Myc[1]. Later studies have supported a role in cancer suppression and revealed functional complexity of alternately spliced and localized isoforms this gene. Several studies have demonstrated frequent attenuation of Bin1 in human cancer, particularly in prostate cancers[2,3], linked to altered apoptotic susceptibility and growth regulation[2,4-13]. Recently, studies in a mouse knockout model generated in our laboratory[14] have demonstrated that Bin1 loss promotes tumor formation in part by promoting immune evasion, an effect that is causally associated with deregulated expression of the immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO)[15].

The classical function of IDO, a ubiquitous oxidoreductase, is in tryptophan catabolism and biosynthesis of the central metabolic regulator nicotinamide dinucleotide (NAD). However, in more recent research, it has been found that the ability of IDO to deplete local concentrations of tryptophan can influence the ability of antigen-presenting cells to activate T cells[16-18]. IDO is upregulated by interferon-γ, which controls the ability of antigen-presenting cells to activate cytotoxic T cells (current speculation is that IDO may have a feedback role since interferon-γ activates immunity). When IDO is activated, tryptophan levels fall and antigen-presenting cells can no longer activate T cells effectively, because in the absence of sufficient tryptophan T cells cannot undergo the cell divisions that are required to become activated. This rather surprising role for IDO as an immunosuppressor has accumulated significant support in the literature, perhaps most dramatically illustrated by the ability of a bioactive IDO inhibitor (1-methyl-tryptophan or 1MT) to cause rejection of allogenic but not syngeneic mouse concepti[16].

It has been known for many years that patients with solid tumors have reduced levels of serum tryptophan and that tumor resection corrects the deficit[19-22]. The meaning of this effect has been unclear however. As the only systemic enzyme that degrades tryptophan, IDO has been implicated for depletion of serum tryptophan in cancer patients[21]. Recently, IDO was shown directly to be overexpressed in many cancers, including prostate cancers, and a consequence of its overexpression was shown to be a reduction in T cell-mediated tumor immunity[23].

Putting this information together, our work suggests that Bin1 limits IDO expression, such that Bin1 attentuation during prostate tumorigenesis promotes progression by stimulating elevation of IDO and immune evasion.

One implication of this model is that inhibiting IDO activity might reverse the consequences of Bin1 loss and IDO elevation in cancer, thereby encouraging immune recognition and tumor rejection. We have tested this idea using a known bioactive inhibitor of this enzyme (1-methyl-tryptophan or 1MT) as well as a novel inhibitor

isolated in our laboratory that is more potent and soluble (methyl-thiohydantoin-tryptophan[15]. We found that, on its own, IDO inhibition with either inhibitor produced only limited growth inhibition in a transgenic mouse model of breast cancer (MMTV-neu mice) that is well-accepted and well-suited to drug response studies (similar results have been reported recently in other models by two other groups[23,24]). The limited effects of IDO inhibition were not enhanced by co-administration of immune activating cytokines such as IL-12 or interferon-γ In contrast, we found that the combination of an IDO inhibitor with paclitaxel, cisplatin, or certain other cytotoxic drugs (that are inefficacious by themselves in the model) produced massive tumor cell deaths and regressions within a two-week treatment period[15]. Host immunity was implicated in these responses, as drug synergy was recapitulated in syngeneic (immunocompetent) mice engrafted with tumor cells, but not in nude mice where T cell immunity is impaired.

One question raised by this line of work is whether the combination principle is applicable to other tumors. This question is important to entertain, particularly because the work on Bin1 and IDO to date suggests that these genes may act in a mechanism or pathway that modifies cancer progression. Cancer modifier pathways can be highly tumor-selective in nature (e.g. *Mom1* in colon cancer). We do not have preliminary evidence that IDO inhibitors will be as active in prostate models as compared to breast models of cancer. For these reasons, we proposed to explore the hypothesis that IDO inhibition could increase chemotherapeutic efficacy in a mouse model of prostate cancer. Our faculty collaborator Janet Sawicki was engaged in generating a derivative of the TRAMP model which was strategized to express a prostate-specific luciferase gene driven by the human PSA promoter (termed TRAMP/PSA-luc mice). In principle, this model would be well-suited to therapeutic studies since it would allow tumor growth to and therapeutic response to be easily monitored by non-invasive bioluminescence imaging. Xenograft models are inappropriate to test IDO-based therapies because of the absence of host immunity. We proposed a high risk-high payoff project based on sound concepts rather than direct preliminary results.

Central Aim. We sought to test the hypothesis that combining IDO inhibition with cytotoxic chemotherapy may safely enhance antitumor efficacy against prostate tumors.

Model System. The model system proposed was a variant TRAMP mouse model of prostate cancer[25] engineered with a prostate-specific luciferase transgene. In principle, this model would allow tumor growth and therapeutic response to be monitored by bioluminescence imaging.

BODY

Summary. Our objectives were to assign tumor-bearing mice to control and drug treatment groups and to compare tumor response after control or experimental therapy by bioluminescence imaging:

Objective I. Generate a colony of TRAMP/PSA-luc mice by breeding.

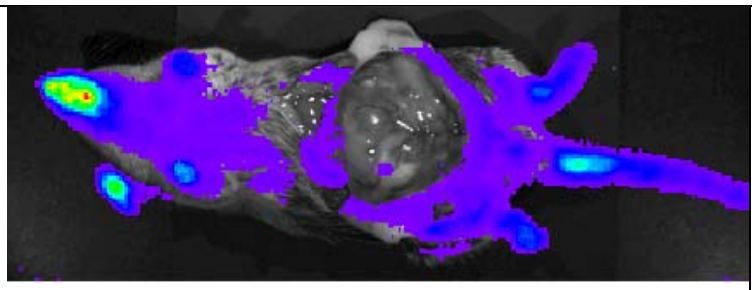
Objective II. Assign tumor-bearing mice to control and treatment groups. Monitor tumor formation by bioluminescence imaging.

Objective III. Assess the effect of IDO inhibition on the therapeutic response to cytotoxic therapy. Perform efficacy and survival tests. Examine tissue histologies from control and treatment groups.

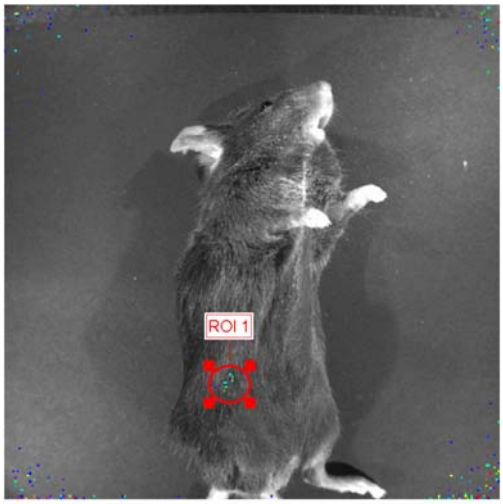
Unfortunately, a variety of technical difficulties in the development and use of the model system, as well as two other model systems that were explored, prevented any progress on addressing the central question under study.

Difficulties in the operation of the originally proposed model. In preliminary work with our collaborator Janet Sawicki (LIMR), we discovered that the PSA-luc transgene to be moved into TRAMP for prostate-specific bioluminescence actually lacked specificity for the prostate. In the PSA-luc transgenic mouse strain that was constructed, the expression of the PSA-luc transgene was expressed not only in prostate but also in many other tissues, making the system useless for our purpose (Fig. 1). This problem prevented us from accomplishing Objectives I, II, and III as originally proposed.

Fig. 1. Background fluorescence in the TRAMP PSA-luc model. A transgenic mouse harboring a large prostate tumor (dark central sphere) was examined on a Xenogen bioluminscence imager using standard methods.



Difficulties in the use of other luciferase-based systems to generate prostate tumors. To address this problem, we explored an alternative experimental strategy in which we orthotopically implanted mouse prostate tumor cells into the prostate. Briefly, we reconstituted orthotopic tumors using a malignant mouse prostate cell line termed MPR, which was created by transformation of *p53–/–* prostate cells with activated c-*myc* and mutant *H-ras* oncogenes. MPR was created and characterized by Dr. Timothy Thompson in the Department of Urology at the Baylor College of Medicine (Houston TX), who kindly provided this cell line[26]. So that we could monitor tumor cells *in vivo* by bioluminescence imaging, we generated by stable transfection a derivative of MPR that constitutively expressed luciferase (MPR-luc cells). To generate prostate tumors, MPR-luc cells were injected orthotopically into one of the lateral lobes of the mouse prostate from a syngeneic mouse, as described previously[26]. Through this alternate experimental design, we sought to address the tasks comprising Objective III. After implantation of MPR-luc cells in the prostate of five male C57/BL6 mice, we could image tumors that arose through bioluminescence at days 3 and 10, however, the bioluminescence signal in all animals was abolished by 17 days without any therapeutic treatment (Fig. 2).



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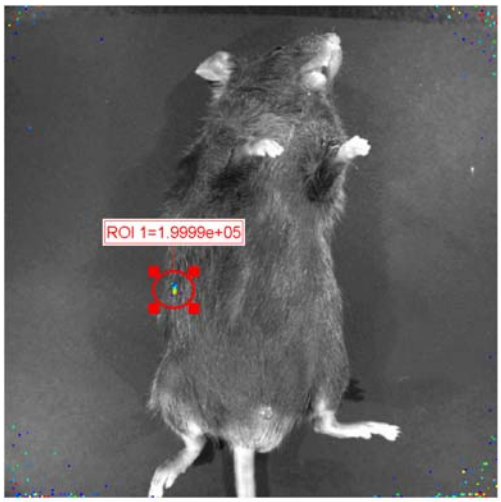

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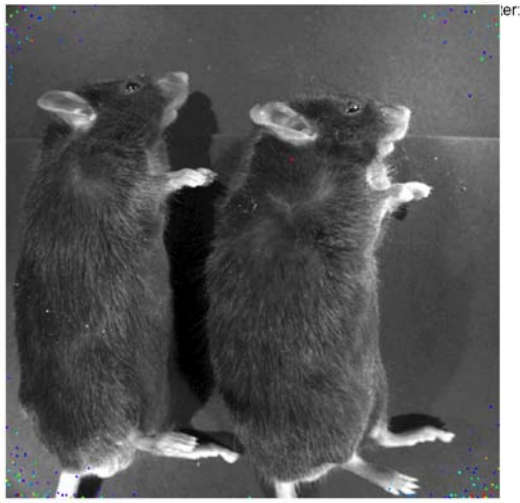

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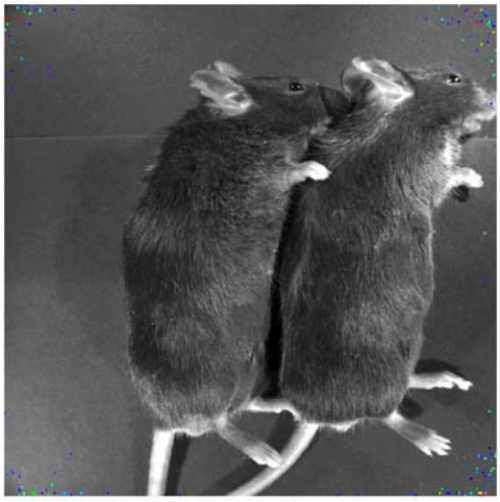

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Fig. 2. Loss of bioluminscence following orthotopic introduction of luciferase-tagged MPR prostate cancer cells. MPR cells (10e7) expressing a luciferase transgene pCAG-luc were introduced on 4/18/05 by orthotopic injection into the prostates of syngeneic C57BL/6 mice. Mice were examined by bioluminscence imaging using standard methods on the following dates indicated. Tumor cells are detectable on 4/25/05 but undetectable on later dates.

Although we did not appreciate it at the time, subsequent experiments performed over a period of several months with a variety of luciferase-expressing clones revealed that this situation was a frequent problem. In this case, excision of the prostates from euthanized animals for fixation, tissue sectioning, and H&E staining revealed no evidence of tumor cells in the prostate. The basis for this phenomenon was not determined and we abandoned this strategy to pursue a different model system for testing the effect of IDO inhibitors on chemotherapy.

In a second alternative design, we used the C2 mouse prostate tumor cell line derived by establishment of an autochthonous TRAMP tumor in tissue culture[25]. C2 cells were kindly provided by Dr. Norman Greenberg at the Fred Hutchinson Cancer Research Center (Seattle WA). As before, we transfected C2 cells with a luciferase vector and selected stable derivatives with high-level transgene expression by standard methods (C2-luc cells). In a variation of previous experiments, we injected C2-luc cells subcutaneously into the intrascapular region of male C57/BL6 mice. After implantation, we were able to successfully image tumors that arose *in vivo* by bioluminescence imaging, starting at day 3. However, as we had seen with MPR cells, by day 10 the bioluminescence signal had been abolished and there was no palpable tumor present at the site of injection (Fig. 3).

In an effort to select for tumor cells which escaped this process, we repeated experiments with MPR-luc and C2-luc cells in which animals were monitored for at least one month after injection with weekly palpatation for tumors. At the end of this period, only one animal exhibited a tumor, indicating that the efficiency of stable tumor outgrowth was too poor to be useful. These difficulties in establishing the model system which was proposed in Objective I were not overcome during Year 1 of the project.

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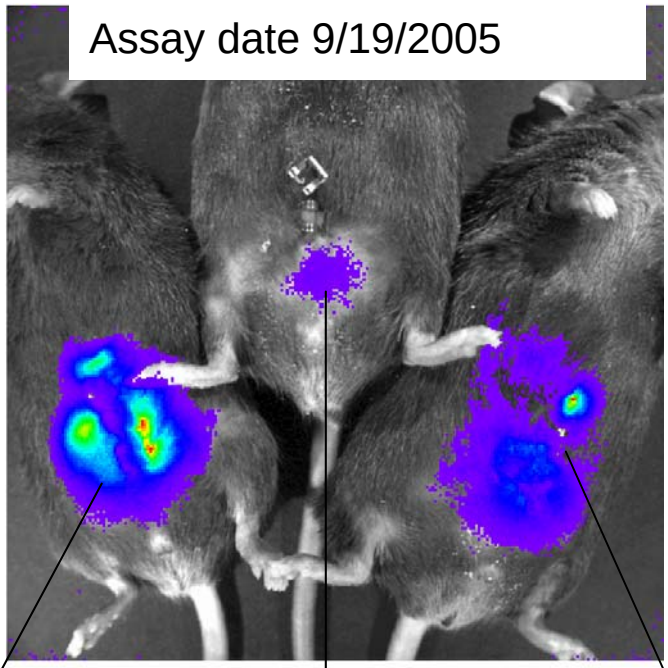
Fig. 3 (page following). Loss of bioluminscence following orthotopic introduction of luciferase-tagged C2 prostate cancer cells. C2 cells (10e7) expressing high levels of luciferase from a pCAG-luc transgene were introduced on 9/12/05 by orthotopic injection into the prostates of syngeneic C57BL/6 mice. Mice were examined by bioluminscence imaging using standard methods on the following dates indicated. Tumor cells are detectable on 9/19/05, less detectable on 9/26/05, and almost undetectable on 10/4/05, despite empirical evidence of tumor outgrowth.

Assay date 9/19/2005



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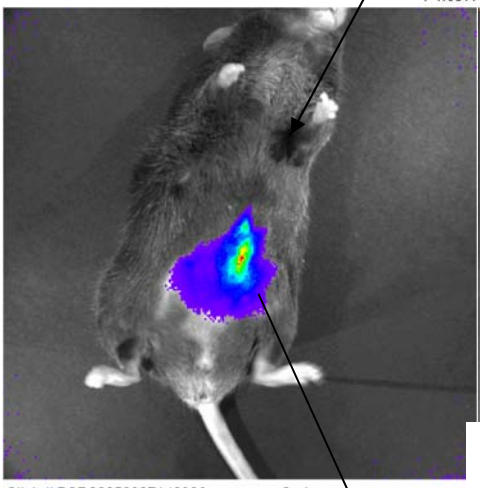

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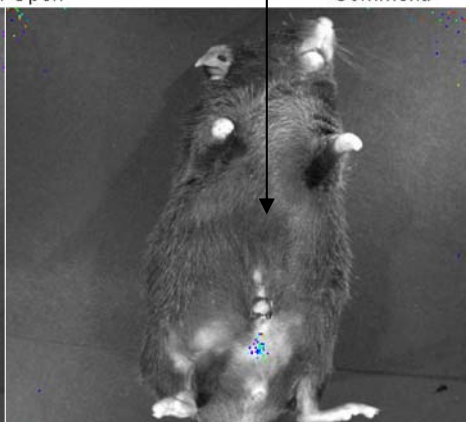

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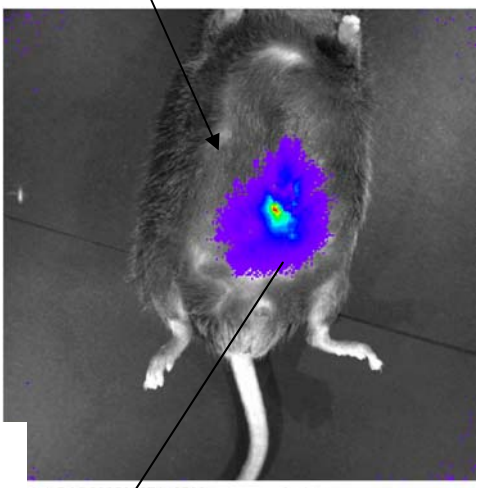

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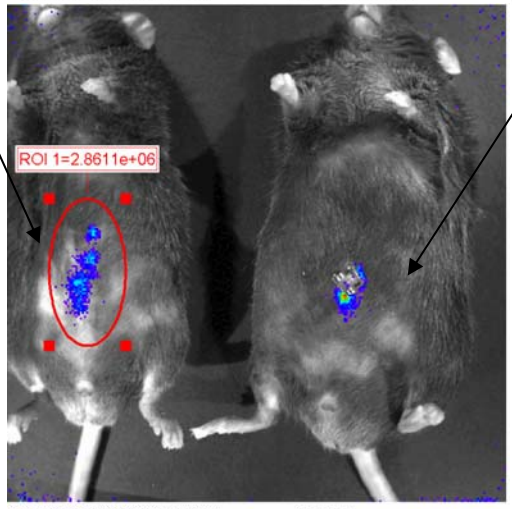

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KEY RESEARCH ACCOMPLISHMENTS

None.

REPORTABLE OUTCOMES

The *in vivo* use of luciferase-expressing tumor cells in immune competent mouse models of prostate cancer may be problematic for allograft studies of cancer pathophysiology and experimental therapeutics.

CONCLUSIONS

A fatal pitfall in the model system to be used for the study was encountered early in the project. Two alternate tumor models that were explored also exhibited fatal pitfalls; these models were each based on engrafting luciferase-expressing cells into the prostate or under the skin of mice. In each case, tumors initially arising in the immunocompetent animals that must be used to test IDO-based experimental therapeutics almost invariably regressed. Experience with these models raised serious concerns about the utility of luciferase-expressing prostate tumor cells for bioluminescence-based studies of prostate cancer pathophysiology and therapeutic response.

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